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# Apoptosis in Cardiac Disease

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For most of this century, tissue death and the cells involved were, with rare exceptions, made synonymous with necrosis. Serious challenge to this overarching dogma began with the presentation of strong evidence for a second form of cell death named apoptosis, characterized by a variety of morphological and biochemical differences from necrosis (1,2). Wide acceptance of this novel concept of nonnecrotic cell death was temporarily delayed by a vigorous dissent from a large number of biochemically oriented scientists who insisted that their term of "programmed cell death" was more suitable and that apoptosis was a flawed concept (3). Although both terms (apoptosis and programmed cell death) currently remain in use by investigators, and lively debate continues as to whether they are the same or different conditions, increasingly wide acceptance of the concept of apoptosis has now established it as importantly different from necrosis, in large measure because it explains many examples of unequivocal cell death that simply cannot be legitimately diagnosed as necrosis. This certainly is true for fetal and postnatal morphogenesis of a variety of tissues and organs, including the human heart (4,5). Based on current knowledge, it is unwise to use apoptosis and programmed cell death as interchangeable terms, but there is clearly substantial overlap between them.

Most of the original morphological descriptions of apoptosis remain valid and include the following. Apoptotic cells either shrink or do not change size, whereas necrotic cells typically swell. The plasmalemma of apoptotic cells remains intact, but this external cell membrane of necrotic cells promptly ruptures. Most organelles (mitochondria, sarcoplasmic membrane, and so forth) of apoptotic cells remain morphologically preserved, whereas these same structures soon rupture or disintegrate in necrosis. The nucleus of apoptotic cells typically partitions its chromatin marginally and then the nucleus is cleaved into separate membrane-bound particles (apoptotic bodies), while the necrotic nucleus randomly clumps its chromatin as the nucleus itself disintegrates. Histologically, necrosis is characterized by all the consequences of cellular disintegration including tissue acidosis and the rapid chemotactic attraction of lymphocytes and neutrophils. By contrast, apoptotic cells quickly exteriorize molecules of phosphatidylserine from their normal internal location, thereby signalling macrophages (and even like cells such as neighboring myocytes) to engulf them, leaving the apoptotic focus of cell death conspicuously devoid of inflammation. If phagocytosis fails, for whatever reason, apoptotic cells eventually disintegrate, releasing their internal contents, and then become indistinguishable from necrosis. Sites of apoptosis in the myocardium, where local capacity for phagocytosis has not been exceeded, resemble a well-tended garden (Figure 1), whereas the morphological chaos of necrosis morphologically resembles a garbage dump (Figure 2).

Cardiologists were late in embracing the concept of apoptosis, although immunologists, hematologists, oncologists, and developmental biologists quickly grasped its importance. Before I wrote on normal and abnormal consequences of apoptosis in the human heart only a few years ago (5), I carefully examined the published index from the annual scientific sessions of both the American Heart Association and American College of Cardiology for the previous 5 years, and there was not a single paper indexed for apoptosis. That has now changed with a continuing growth of cardiologic interest in apoptosis each year.

In the present discussion of apoptosis in cardiac disease, I will examine its role in several different arbitrarily chosen examples based upon personal experience with them. For readers not acquainted with apoptosis, the two major approaches for studying its role in cardiac disease are ones based primarily in biochemistry and others dealing with morphology. For experimental studies in rats or with cell culture or liquid blood, it is not difficult to combine biochemistry and morphology; but this is not easily done with human tissue, especially if one is to study myocardial cytology and histology. Flow cytometry and gel elctrophoresis cannot be performed with tissue samples without liquifying or emulsifying them. Electrophoretic patterns, for example, tell us nothing about the histologic

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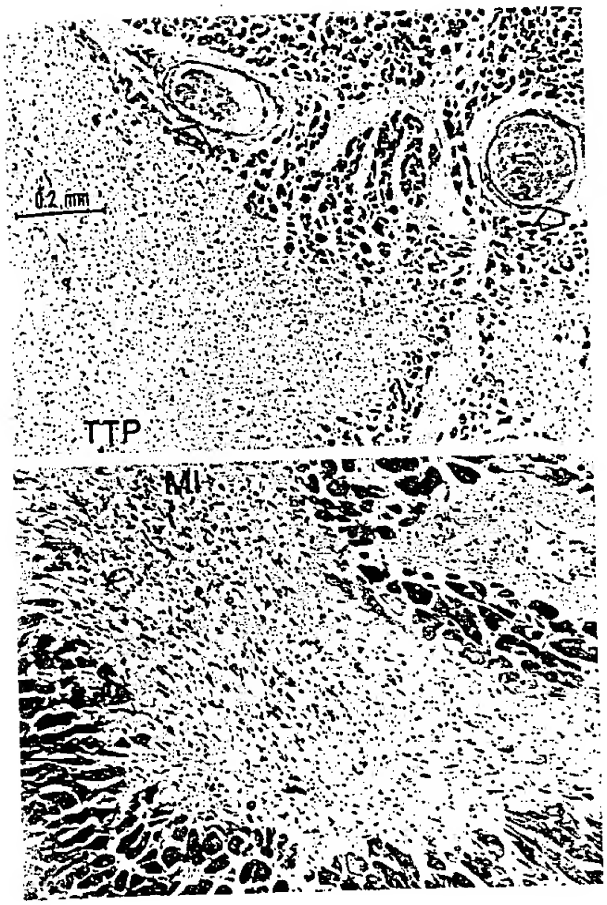


Figure 1. An illustration comparing the similarity between a myocardial focus of apoptosis in thrombotic thrombocytopenic purpura (TTP) and myocardial infarction (MI), in two different patients. Note the sharp demarcation between the pale areas of apoptosis and adjacent unaffected myocardium. Two open arrows mark small arteries occluded with platelets. (See text for discussion. Goldner trichrome stain in both examples. Magnification here and all subsequent photomicrographs is indicated with a reference bar and refers to both sections of a figure unless otherwise indicated.)

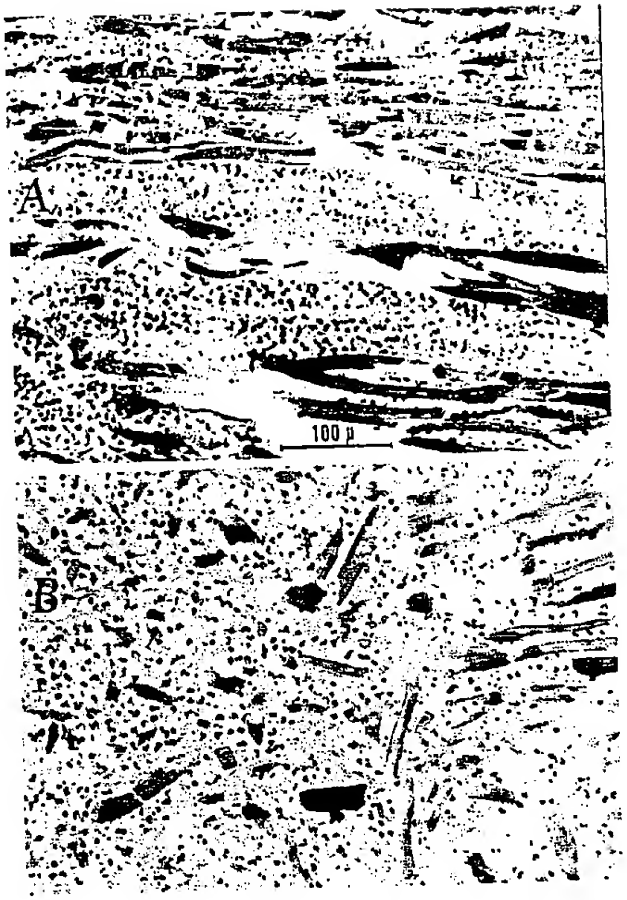


Figure 2. The histological chaos typical of necrosis in acute myocardial infarction is shown here from two areas of a 62-year-old man's heart. There is extensive infiltration with neutrophils and lymphocytes plus total disintegration of myocardial structure. Note the contrast with the appearance of apoptotic infarction in Figure 1.

or cytologic picture of acute myocardial infarction. A major asset for bridging the gap between these technologies has been the development of immunohistochemical methods for selective staining of apoptotic nuclei and apoptotic bodies, the latter usually representing membrane-bound nuclear fragments. For this purpose, the most useful method for me has been the TUNEL stain developed by Gavrieli et al (6) that identifies any nucleus containing broken strands of DNA. Now a rapidly growing number of ancillary approaches are available and utilize selective immunohistochemical demonstration of various promoters or inhibitors of apoptosis.

A frequently encountered criticism for the morphological diagnosis of apoptosis is an adamant insistence that apoptosis can be diagnosed only if multiple forms of evidence are encompassed, usually meaning gel electrophoresis and other biochemical measures, and that a single criterion such as selective staining of an apoptotic nucleus is inadequate. This criticism is without merit for the false premise it includes, ie, that the morphological diagnosis is one-dimensional and considers only the nuclear staining. Any focus of apoptotic cell death in human myocardium has at least the following multiple diagnostic morphological features: 1) an apoptotic focus is distinctively separated by a sharp visible boundary from viable myocardium; 2) the apoptotic focus has a homogenous appearance, totally devoid of any evidence of inflammation (ie, no neutrophils or lymphocytes); 3) the focus is filled with relatively intact myocytes mixed with fibroblasts, macrophages, and neural and vascular elements, any number of which may stain positively for apoptosis; 4) throughout the apoptotic focus there are numerous macrophages containing either a few or many ingested apoptotic bodies; 5) in some apoptotic areas, large groups of apoptotic bodies aggregate in sheets or pools in the intercellular space, owing to local over-

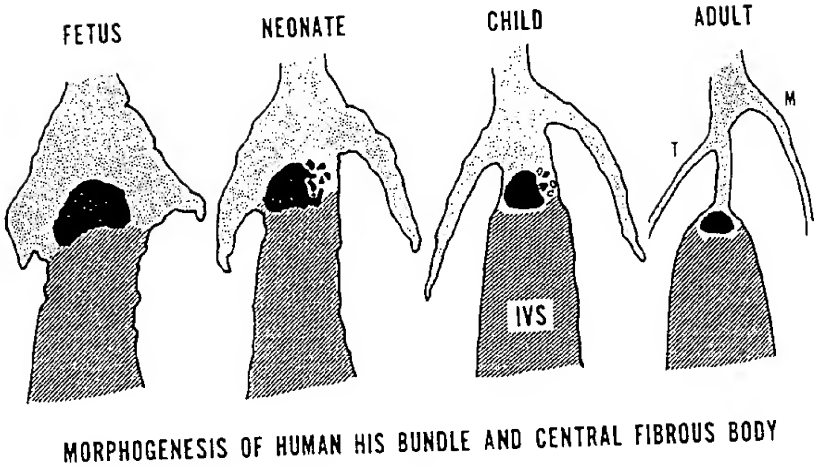


Figure 3. This cartoon depicts the normal postnatal changes during morphogenesis of the His bundle. There can be either normal or abnormal consequences of such changes, as discussed in the text. IVS = interventricular septum; T = tricuspid valve; M = mitral valve.

whelming of phagocytic capacity; 6) nearly every apoptotic focus also contains intermixed nonapoptotic cells, and the negative staining of their nuclei makes a useful contrast with the TUNEL-positive nuclei of the apoptotic cells. Some have argued that the typical apoptotic focus in human myocardial infarction simply represents the "healing phase of infarction," and in older literature that was often claimed; but the presence of histochemical evidence of recent cell death, plus the presence of macrophages filled with apoptotic bodies, render that old concept no longer tenable.

## NORMAL AND ABNORMAL CONSEQUENCES OF APOPTOSIS IN THE HUMAN HEART

Unlike necrosis, the results of which are nearly always destructive, apoptosis may be either beneficial or harmful depending upon coexisting circumstances. In the myocardium, necrosis not only subtracts those myocytes directly involved, but because of the prompt breakdown of both the internal contents and the external cell membrane (plasmalemma), necrotic cells also generate an additional toxic situation within the surrounding extracellular space. Furthermore, the rapid invasion by lymphocytes and neutrophils has its own noxious influence upon local surviving myocytes. By contrast, apoptotic cell death is devoid of nearly all these secondary harmful features of necrotic cell death. The plasmalemma remains intact after the apoptotic myocyte dies, thus evading both toxifying its environment or attracting secondarily harmful leucocytes. However, there are conditions in which apoptosis goes awry. These are usually the result of derangement in the exquisite balance of many different factors either promoting or inhibiting the apoptotic process (7,8), factors in a rapidly growing volume of biochemical substances either normally occurring in nature or introduced by disease or by pharmacological agents.

The yin and yang character of apoptosis can be illustrated by what happens in the postnatal development of the cardiac conduction system and/or what happens in the postnatal right ventricle. The sinus node, AV (atrioventricular) node, and His bundle all undergo major anatomical transformation after birth, and much or most of this happens by programmed destruction mediated by apoptosis (4,5). The His bundle changes from a relatively "enormous" shaggy structure in the fetal heart to a smoothly outlined slender tube normally present in adolescence and adult life (Figures 3 and 4). There are reasons to believe that this postnatal morphogenesis is electrophysiologically safer and promotes electrical stability of the heart. Conversely, persistence of the fetal morphology in the adult AV node and His bundle is often associated with sudden unexpected death (9,10). The fetal sinus node is composed primarily of myofibril-poor small round or oval myocytes (P cells), whereas the adult sinus node has far more collagen within its normal periarterial composition. The P cells are either transformed into transitional cells (slender elongated myofibril-rich myocytes), or the former are replaced by the latter (Figure 5). In either case apoptosis plays a significant role in this postnatal morphogenesis of the human sinus node as well.

The fetal and early postnatal size and thickness of the right and left ventricles are about the same, unsurprising since both ventricles have similar vascular resistance to work against during fetal development. This situation quickly changes after birth when the pulmonary circula-

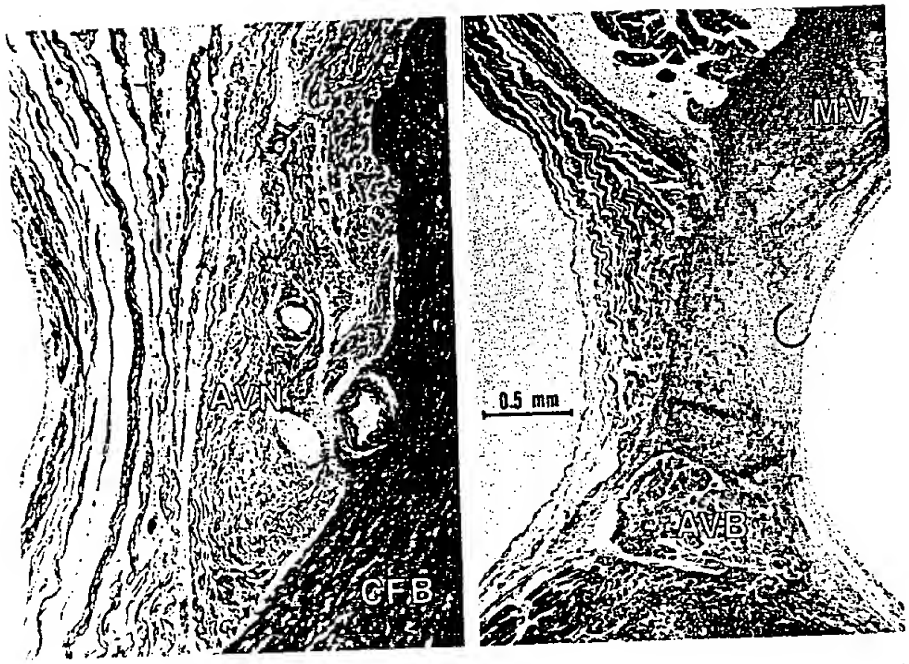


**Figure 4.** Normal histological appearance of the adult human AV node (left, marked AVN) and His bundle (right, marked AVB) is illustrated here. Note the smooth contour of the AVN as it lies upon the central fibrous body (CFB), and the smooth outline of the His bundle deeply placed in the CFB: the CFB normally forms the anchor of the mitral valve (MV). (See also Figure 3.)

tion opens and both the ductus arteriosus and the foramen ovale normally close. The obvious major difference in size and thickness of the two ventricles of an adult heart is conventionally attributed to steady growth of the left ventricle because of its newly acquired greater peripheral resistance. By comparison, perhaps the right ventricle simply fails to grow (11). However, elegant experiments have demonstrated that the murine right ventricle rapidly undergoes active involution with cell death during the early postnatal period, and that this is accomplished by apoptosis that does not significantly take place in the left ventricle (12). The concept that the postnatal right ventricle fails to grow seems simplistic for another reason considered teleologically: there are many examples in nature wherein unused tissue in the body is systematically removed rather than only left alone, "disuse atrophy" being a familiar occurrence. And removal of tissue this way is nearly always mediated by apoptosis, as it is in the postnatal right ventricle.

It is understandable why precise control of apoptosis in the sinus node, AV node, His bundle, and right ventricle is essential in order that there be just the right amount and not too much, for apoptotic myocytes are just as dead as necrotic myocytes. Pathological effects of too much apoptosis destroying either the sinus node or AV node or the His bundle, or all three, have already been described, and some of these will be examined in the other categories to be presented here. The same is true for the postnatal right ventricle, with excessive focal degeneration or massive overall destruction of the right ventricle representing known clinical entities, as will also be discussed. As may be expected, each of these examples of excessive or misdirected apoptosis is associated with arrhythmogenesis, impaired AV conduction, and, too often, with sudden unexpected death. There is a paradoxical irony inherent in postnatal apoptotic morphogenesis in the human heart, on the one hand being a perfectly normal situation, which on the other hand can sometimes become abnormal and troublesome when its exquisite regulation by promoters and inhibitors is unbalanced. As a discouraging corollary, some morphologists still today too often refuse to interpret focal apoptotic cell death as important "because it is not necrosis."

## THROMBOTIC THROMBOCYTOPENIC PURPURA

There are several surprising but useful lessons for all physicians that come from learning more about this uncommon hematological disease, particularly as concerns its involvement of the heart. Thrombotic thrombocytopenic purpura (TTP) is characterized clinically as episodic widespread occlusion by platelet masses within small arteries and capillaries. An associated paradox included in the name of this disease comes about because so many platelets are "consumed" by this extensive multifocal thrombosis that a deficit of circulating platelets coexists, resulting in purpura and other bleeding problems.

Virtually every organ is affected by the episodic bouts

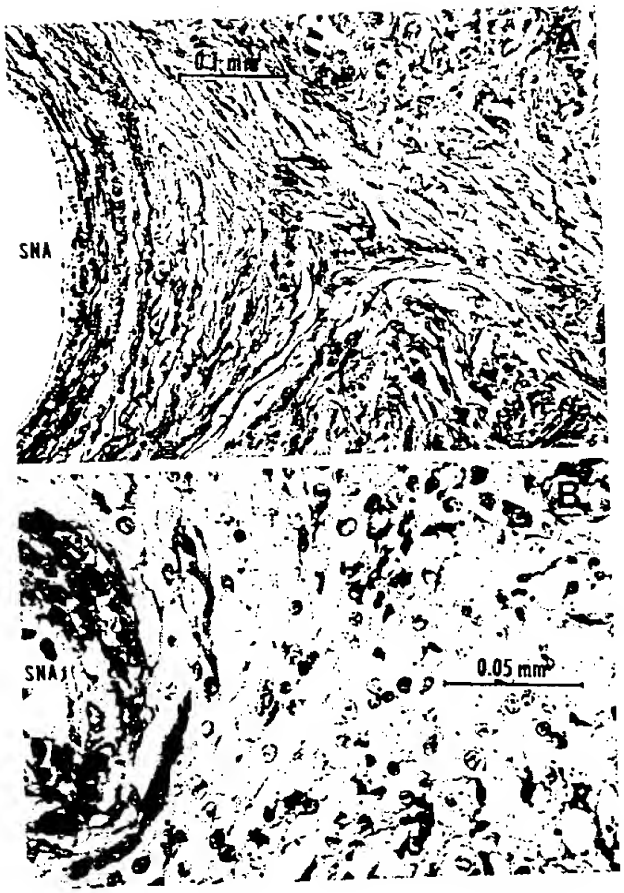


**Figure 5.** Changes in the human sinus node during postnatal morphogenesis are illustrated with examples from a normal adult (above) and infant (below). There is much more collagen in the normal adult sinus node (green in the Goldner stain), but predominantly P cells in the infant (see text for discussion). Human sinus nodes normally contain a conspicuous central artery (SNA) about which the node is organized. Apoptosis plays a major role in the postnatal morphogenesis of the human sinus node, AV node, and His bundle.

typical of TTP, but notably affected are the kidneys, brain, adrenals, and the heart. My interest in TTP was first kindled when I encountered 2 patients with TTP who died with complete heart block (13). At postmortem examination there were not only widespread platelet occlusions of small coronary arteries (including blood supply to the conduction system) but also noninflammatory focal myocardial degeneration throughout the heart. The His bundle was virtually transected by this degeneration (Figure 6), reflected clinically by fatal heart block documented electrocardiographically. These findings were later confirmed by pathologists at Johns Hopkins in a series of their own TTP patients in whom they saw not only focal noninflammatory degeneration throughout the myocardium but also specifically the same focal degeneration in the conduction system (14). Despite those reports, a study from the Armed Forces Institute of Pathology denied the significance of such findings by pointing out that there was no necrosis and, ergo, no clinical significance (15).

This led me later to examine the possibility of focal apoptosis in TTP as the basis for the many small regions of myocardial degeneration (16) already demonstrated to be associated with concomitant narrowing and occlusion of small coronary arteries by platelet aggregations (Figure 1). All the focal myocardial degeneration characteristic of fatal cases of TTP proved to be noninflammatory apoptotic cell death, and nowhere was there any example of necrosis. Some small foci of intramyocardial hemorrhage (not typical of focal apoptosis) were best attributable to the thrombocytopenia plus the frequent occurrence of fibrinoid degeneration of the walls of small arteries and capillaries also typical of TTP. All of these many apoptotic foci throughout the myocardium add up to an inevitable subtraction from the volume of normal myocardium and inotropic capacity, suggesting that myocardial insufficiency must eventually be encountered among some survivors of numerous bouts of active TTP. Crucially located apoptotic foci, eg, in the His bundle (Figure 6) or the sinus node would directly lead to significant electrical instability, arrhythmias, syncope and sometimes sudden death from that cause.

With so much myocardial involvement in TTP one may ask why did not some of its effect end in necrosis? The probable answer lies in the fact that TTP affects almost exclusively small arteries and capillaries, and usually does so in an episodic fashion. It seems likely that the magnitude of injury with even multifocal occlusion of small coronary arteries would remain within the local phagocytic capacity at each apoptotic focus. As long as the local capacity for phagocytosis is not exceeded, there would be no cell breakdown typical of necrosis. The special significance of these several considerations applies particularly to a better understanding of acute myocardial infarction, as will be discussed next.

## THE VARIABLE MORPHOLOGIC COEXISTENCE OF APOPTOSIS AND NECROSIS IN MYOCARDIAL INFARCTION: SIGNIFICANCE FOR UNDERSTANDING ITS PATHOGENESIS, CLINICAL COURSE, DIAGNOSIS, AND PROGNOSIS

In elegant experiments utilizing coronary ligation in rats, Kajstura et al (17) demonstrated that cell death in the first couple of days of myocardial infarction was entirely by apoptosis, in the next few days a mixture of apoptosis and necrosis, and all cell death after that was essentially by necrosis. From my own examination of 77 human hearts

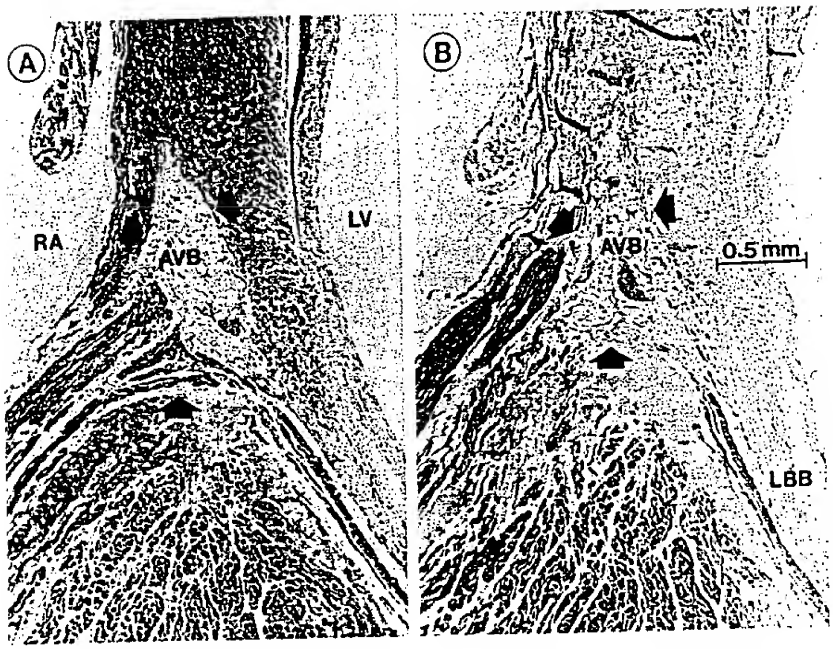


Figure 6. These photomicrographs demonstrate the apoptotic destruction of two closely adjacent sections taken from the His bundle of a patient with thrombotic thrombocytopenic purpura dying from electrocardiographically documented complete heart block. Small black arrows mark the area where His bundle would normally have been. RA = right atrium; LV = left ventricle; LBB = left bundle branch.

of individuals dying of acute myocardial infarction, I found that both apoptosis and necrosis were always present in every case (18). In addition to possible species differences affecting murine and human comparisons, there is a conspicuous and important difference between the effect of experimental acute coronary ligation and what probably happens in human myocardial infarction.

There is no single clinical picture or course applicable to all human myocardial infarctions. On the contrary, the frequency of waxing and waning of symptoms and findings so often seen early in human myocardial infarction has led to the imaginative description of a "stuttering" onset (19). Many factors contribute to this initial clinical instability, including the nature of the coronary obstruction (spasm may wane, platelet clumps can disintegrate, partial or intermittent unhinging of a fragment of plaque can occur), or the level of myocardial metabolic demand (compounded by many autonomic and neurohumoral influences upon the heart); arrhythmias can come and go, the level and effectiveness of available collateral circulation can fluctuate, and countless other events such as fever, medications, and high or low blood pressure can each and all continually change the clinical picture. Accordingly, it is understandable why both the exact time of onset and the subsequent progression of these human events are seldom susceptible to optimal timing analysis. Some valuable clues can be had from a careful history obtained from the patient, from electrocardiography and/or echocardiography or from clinical hemodynamic status, and of course from coronary arteriography. But even with all these, the clinician is too often confronted with paradoxical events and frustrating variation of clinical status that do not "fit" with expectations.

Several decades ago it was first proposed that variation of serum enzymes during acute myocardial infarction could be useful in both the diagnosis and prognosis in such patients (20). The fundamental logic was simple and appealing: 1) all myocardial infarction was characterized by necrosis (no real consideration of other forms for cell death was entertained); and 2) necrotic cells disgorged all their contents, including certain enzymes measurable in the blood. The first serum enzyme selected for this purpose was glutamic oxalacetic transaminase, but this was too often distorted by the same enzyme being released from skeletal muscle, liver and other sources. The same fate befell lactic dehydrogenase. A search for a more "cardiac-specific" enzyme led us transiently to myoglobin, then into creatine kinase, then the MB fraction of creatine kinase, and now to several different troponins, all being normally intracellular enzymes or markers. Consequently, although all these markers are predictably released from necrotic cells, they cannot be expected to be released by the apoptotic cells, which are equally dead.

So much interest is currently placed in the accuracy of timely diagnosis and prognosis in patients with "acute coronary syndromes" that it is disappointing that the methods still depend so much upon measurement of markers in the blood. All such studies continue necessar-

ily to assume that cell death in myocardial infarction is always necrotic. In this context it is significant that a recent study found that there was little prognostic advantage to be gained from measuring troponins compared with subforms of creatine kinase (21). This would suggest that both are equally reflective and selectively diagnostic of "myocardial injury," but of course still *only* that due to necrosis. The fundamental flaw in any dependability of serum marker diagnosis of acute myocardial infarction is not its nonspecificity for myocardial damage versus liver, skeletal muscle, and so forth, but that it fails to account for a very important component of every infarct due to apoptosis. And this failure is especially important *early* in myocardial infarction.

Given the universal coexistence of apoptosis and necrosis in acute myocardial infarction in human subjects (18) and the experimental evidence of their sequential development in experimental studies in rats (17), what can be said of any functional interrelationship between these two very different forms of cell death? Can necrosis cause apoptosis? There is no evidence to suggest this, although one can reasonably conjecture that the noxious effects always accompanying necrosis could lead secondarily to apoptosis near the margins of a necrotic area. Can apoptosis cause necrosis? As a direct action, the answer is no, but apoptotic cells and apoptotic bodies retain their membrane integrity only for a finite time, usually ending in an effective phagocytosis either by macrophages (the professional phagocytes) or by like cells (surviving neighboring myocytes). If phagocytosis fails for whatever reason, then both apoptotic cells and apoptotic bodies eventually break down, release their own internal contents, and become indistinguishable from necrosis. Failure of local phagocytosis can be due to an insufficiency of available macrophages, or to failure of generation of chemical signals by apoptotic cells to attract macrophages, or generation of such signals but for unexplained reasons a failure of the macrophages to receive or interpret them properly. The dynamic interplay between apoptosis and necrosis thus depends in many ways upon the interplay between apoptotic myocytes and phagocytes.

Two other aspects of a possible functional relationship between apoptosis and necrosis in human myocardial infarction warrant comment. First, the title of the otherwise excellent study by Kajstura et al (17) emphasizes an independence of apoptosis and necrosis in their experiments, a commendable caution since they did not demonstrate such dependence per se. But the fact that the sequence of appearance of apoptosis was early and necrosis late does not itself verify their functional independence. As discussed above, given enough time (or size), apoptotic foci can eventually exceed the local capacity for phagocytosis and then everything becomes necrosis. Second, the presence of TUNEL-positive cells within areas of obvious necrosis has been misinterpreted by some as de facto evidence that necrotic cells also have TUNEL-positive nuclei, and ergo the TUNEL positivity is unreliable in indicating apoptosis. But apoptosis and necrosis intermingle in countless morphological configurations in human myocardial infarction, with apoptotic and necrotic areas sometimes completely separate but more often not. Furthermore, one must think in three dimensions and realize that a contiguous area of apoptosis may be either above or below the plane of the necrotic area seen in an 8-micron section of tissue. This is also why it is erroneous to expect that all apoptotic foci in myocardial infarction must lie at the margins of necrosis: There are higher and lower margins as well as lateral ones. And as discussed above, when phagocytosis fails in an apoptotic area, nearly all the dead cells then become necrotic. Finding one or a few of them not yet disintegrated cannot be surprising, even though many of their neighbors may have broken down into a condition of necrosis. One may reasonably conjecture that all cell death in human acute myocardial infarction begins with apoptosis (Figures 1 and 7), as was apparent in the murine experiments (17). The later change may simply evolve to necrosis when local phagocytic capacity is exceeded (Figure 8), but one must remain aware of the many other variables accompanying human cases, as discussed earlier.

## DISEASES PECULIAR TO THE RIGHT VENTRICLE

During most systemic diseases that affect the heart, such as TTP, there is neither predilection for nor sparing of the right ventricle to my knowledge. The fact that the right ventricle normally pumps against much less vascular resistance (the pulmonary circulation) makes it probable that similar levels of ischemia would cause more injury to the harder-working left ventricular myocardium. There have been experiments in which cauterization of the entire free wall of the right ventricle failed to produce right ventricle failure (22). However, there is a unique component of the right ventricle, the crista supraventricularis, that is not a part of the free wall but is attached both to it and to the interventricular septum, permitting this crista to act as the mediator for coordinating right and left ventricular systole in the most efficient manner (23). Damage to the crista supraventricularis leads to tricuspid valvular incompetence and right ventricular failure. There are some special diseases that selectively involve the right ventricle, including the crista supraventricularis, and they are in important ways mediated by apoptosis. The special and selective targeting of the right ventricle by apoptosis represents a form of programmed cell death.

### *Uhl's Anomaly*

This rare but fascinating condition (24,25) is typically seen very early in life but has also been described in adults.

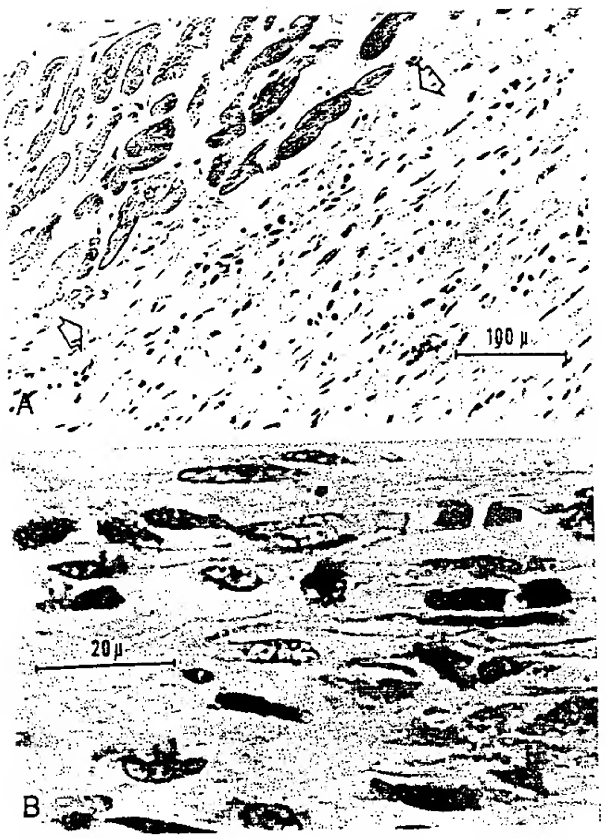


Figure 7. Apoptotic portions of an acute myocardial infarction are shown here from an 18-year-old male with Marfan's syndrome who died with aortic dissection extending into and occluding his right coronary artery. A. Note again the sharp demarcation between apoptosis and normal myocardium, marked with two open arrows. B. Cytology of the apoptotic area is seen at higher magnification (TUNEL stain) where apoptotic nuclei are brown and nonapoptotic nuclei blue. The counterstain is methyl green.

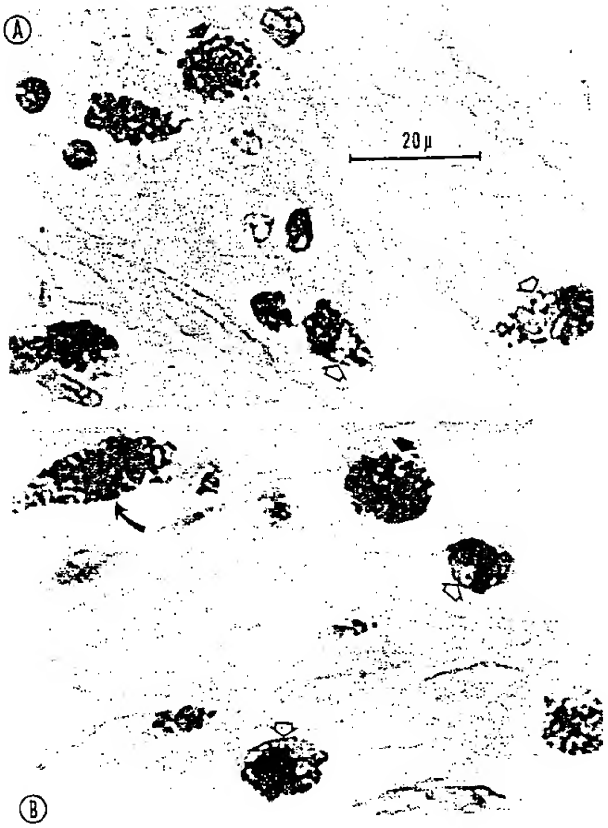


Figure 8. Active phagocytosis of apoptotic bodies by macrophages and by neighboring myocytes is illustrated here (TUNEL and methyl green stains). A. Small black arrows mark macrophages totally packed with apoptotic bodies, while small open arrows mark visible nonapoptotic nuclei (blue) in less packed macrophages. B. The curved black arrow marks a myocyte with many juxtanuclear apoptotic bodies. See text for discussion.

Its etiology has largely been considered as unknown. It is characterized by almost total destruction of the right ventricle by a generally noninflammatory process, leaving the walls of the other three cardiac chambers uninvolved (Figure 9). In some patients there is an associated complete heart block (25), but the usual major functional problem is right ventricular failure ending in death. It now appears that the entire destruction of the right ventricle may be mediated by apoptosis (Figure 10). Small foci of associated infiltration by lymphocytes are probably the consequence of locally overwhelmed phagocytic capacity, but most of the destruction has no associated inflammation.

### *Arrhythmogenic Right Ventricular Dysplasia*

A close counterpart to Uhl's anomaly is the condition of arrhythmogenic right ventricular dysplasia (ARVD), differing mainly in being focal rather than generalized in the right ventricle. ARVD is characterized by multiple sites of fibro-fatty (noninflammatory) degeneration in the right ventricle, particularly in its outflow region. Apoptosis in these ARVD foci was predicted (25) and has now been demonstrated by two different groups (26,27). Why these foci of apoptotic cell death come when they do is unknown, as is why they stop when they do, rather than progressing to complete right ventricular destruction. Although the pathogenesis of the documented bouts of ventricular arrhythmias in ARVD is only incompletely understood, two logical possibilities are that dying right ventricular myocytes may transiently become spontaneously automatic or (more likely) these apoptotic cells become unresponsive (nonconducting) and thereby provide a substrate for development of focal reentrant arrhythmias. Surgical interest in excising or destroying identifiable foci in the right ventricle of ARVD patients must be tempered by the probability of future focal apo-

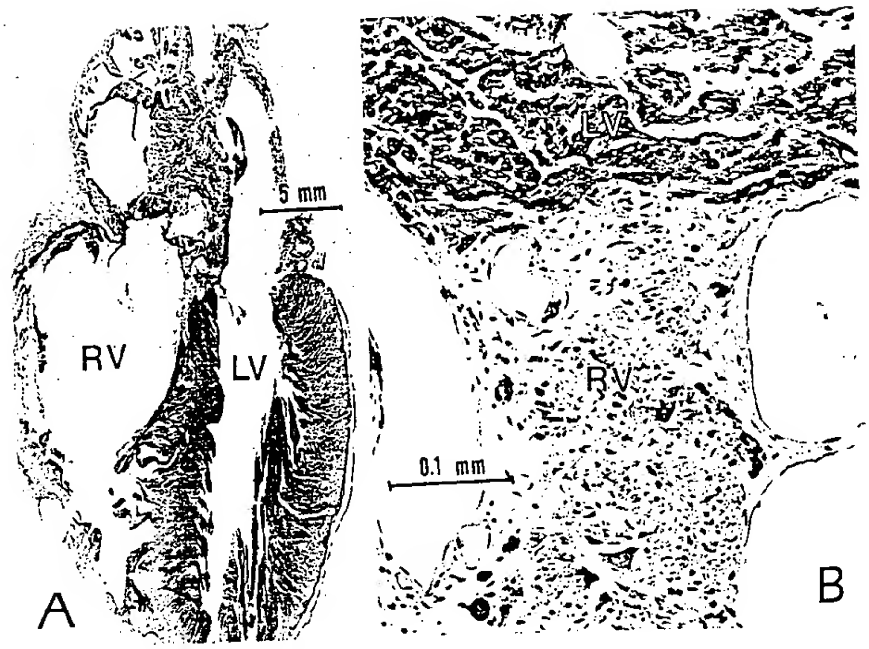


Figure 9. These two photomicrographs are from a fatal case of Uhl's anomaly. A. The low magnification picture shows the thin right ventricular wall, almost devoid of myocytes, with normal myocardium in the three other chamber walls. B. The remarkable demarcation between apoptotic right ventricle (RV) and normal left ventricle (LV) is shown from the apical portion of the interventricular septum of the heart in A. See text for discussion.

ptotic cell death and recurrence of the initial problem that was only temporarily improved.

### *Normal Postnatal Involution of the Right Ventricle*

As discussed previously, the conventional wisdom about postnatal maturation of the heart holds that the right ventricle remains the same size and thickness but that the left ventricle responds to increased vascular resistance by becoming thicker (11). This seems teleologically improbable since nature consistently removes surplus or unused tissue, by "atrophy." That the same may be true for the postnatal right ventricle is supported by experimental studies in rats demonstrating that their right ventricle normally undergoes active involution shortly after birth and that this process is mediated by apoptosis (12). This being the case, Uhl's anomaly can be seen as a completely unchecked continuation of a normal process, in which the beneficial apoptosis normally checked later by anti-apoptotic signals simply failed to happen until the right ventricle was destroyed. ARVD can be seen as an episodic recurrence, focal in distribution in the right ventricle, but one in which the normal postnatal signal somehow transiently resumed (but in focal distribution) and then turns off and on subsequently in unpredictable fashion.

## SYNDROMES OF BRINK AND BRUGADA

A genetically based condition of progressive development of heart block ending in fatal arrhythmias has been described by Brink and colleagues (28,29) in South African families. A similar clinical course has been identified (30) and proved to be associated with gradual apoptotic destruction selectively of the sinus node and AV node but not the His bundle. Intriguingly, nearly all the myocytes in the interatrial septum, representing especially the internodal pathways (31), were also selectively destroyed by apoptosis. There was no destruction in the working myocardium of the two ventricles. Although I am currently unaware of postmortem examination of these regions among the several fatal cases in the South African experience, it is plausible that selective apoptotic destruction of these crucial elements of the cardiac conduction system is the explanation there as well.

The three Brugada brothers (32) have described a condition somewhat akin to both ARVD and the familial problem described in South Africa. The Brugada cases have exhibited ST-segment elevation in right precordial leads and a tendency for syncope and sudden death. I am unaware of postmortem studies of the cardiac conduction system (or of foci in the right ventricular myocardium), but it seems likely that apoptosis also plays a role in this condition.

## THE LONG QT SYNDROME

Knowledge about this heritable genetically based condition has progressed from a very rare discovery in deaf siblings in a Norwegian family, in whom a bizarre elec-

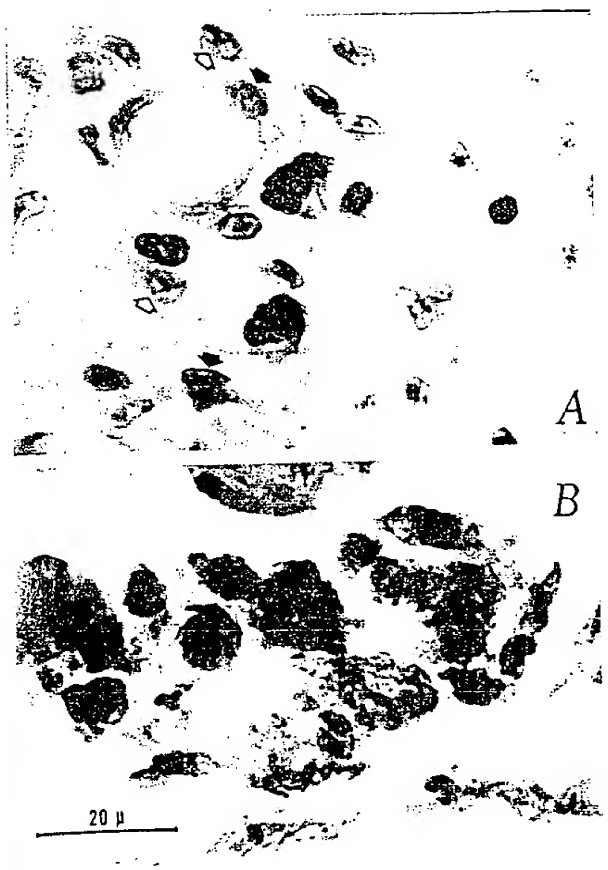


**Figure 10.** Two examples of apoptosis destroying the right ventricle in Uhl's anomaly (same heart as Figure 9) are shown. A. Of a variety of apoptotic cells, two are from endothelium marked with **black arrows**. Nonapoptotic (blue) nuclei are marked with **open arrows**. B. A clumped mass of apoptotic cells.

trocardiogram was present and sudden death occurred in several of them (33), through an almost concomitant recognition that the same condition was present in some normal-hearing children (34,35), and then on to what is now an almost worldwide explosion of knowledge about the long QT syndrome and many related conditions ending in sudden death. There was a temporary lull in interest after the early discoveries because although both groups of original investigators naturally suspected a cardiac death, their autopsy studies revealed no anatomical disease of the heart (33,36). During a population ascertainment study of the problem later conducted among deaf children in the United Kingdom, there was the opportunity to examine the cardiac conduction system of three fatal cases, and a significant cardiac abnormality was present in all three, characterized by focal noninflammatory degeneration of a substantial portion of the sinus node (37). This was prior to the description of apoptosis as a second form of cell death, and predictably some dismissed those findings as lacking significance because "there was no necrosis."

One may question why or how the sinus node might participate in a disease known especially for delayed ventricular repolarization (long QT) and sudden death, now often documented as being due to a particular ventricular arrhythmia beginning with "torsades de pointe" and ending in ventricular fibrillation. However, it was early emphasized that virtually all the original cases exhibited an inordinate sinus bradycardia, especially for children (37). There are a variety of effects secondary to bradycardia that predispose to arrhythmias. But an especially cogent experience was reported by Russian surgeons who developed a seemingly radical approach for treating a series of patients (all of whom had a long QT interval, syncopal attacks, and documented near-fatal ventricular arrhythmias) by surgically excising the sinus node and implanting a permanent pacemaker with remarkably successful results (38). This surgical approach is far less radical than it may seem, actually resembling closely a popular clinical approach utilizing administration of beta receptor blocking agents (for their negative chronotropic effect) combined with permanent pacing (39,40). In fact, the Russian approach has some clinical advantages since a preserved sinus node sometimes dangerously interferes with the electronic pacemaker (41,42). Furthermore, the Russian approach easily permits use of any needed pharmacological therapy without having concern for negative chronotropic effects.

Both light and electron microscopic examination of the excised Russian sinus nodes proved of surprising value by not only confirming earlier findings of focal noninflammatory degeneration in the sinus node but also local intranodal and perinodal cardioneuropathy (43,44). Furthermore, our electron microscopic pictures additionally demonstrated an unusual pleomorphic micromitochondriosis as well as clear examples of apoptosis involving sinus nodal cells (Figure 11). The presence of apoptotic focal destruction in the sinus node of victims dying with the long QT syndrome has now been additionally documented in numerous other (non-Russian) cases (45).

Further comment is warranted about the cardioneuropathy (46) also caused by apoptosis (Figure 12) in and near the sinus node of fatal cases of the long QT syndrome. A dysautonomic basis for the sinus bradycardia has long been considered, possibly mediated by deprivation of sympathetic neural input to that right atrial region, with "unbalanced" stronger sympathetic influence on the left ventricle predisposing to arrhythmogenesis. On this premise, left stellate sympathectomy has periodically been utilized for treating symptomatic long QT patients, but this rationale is flawed by the likelihood that any cardioneuropathy present at any given site may later recur at some other site in or near the heart, further distorting the sympathetic imbalance. However sympathectomy fares in future therapeutic regimes, it is clear that focal noninflammatory degeneration (Figure 12) of

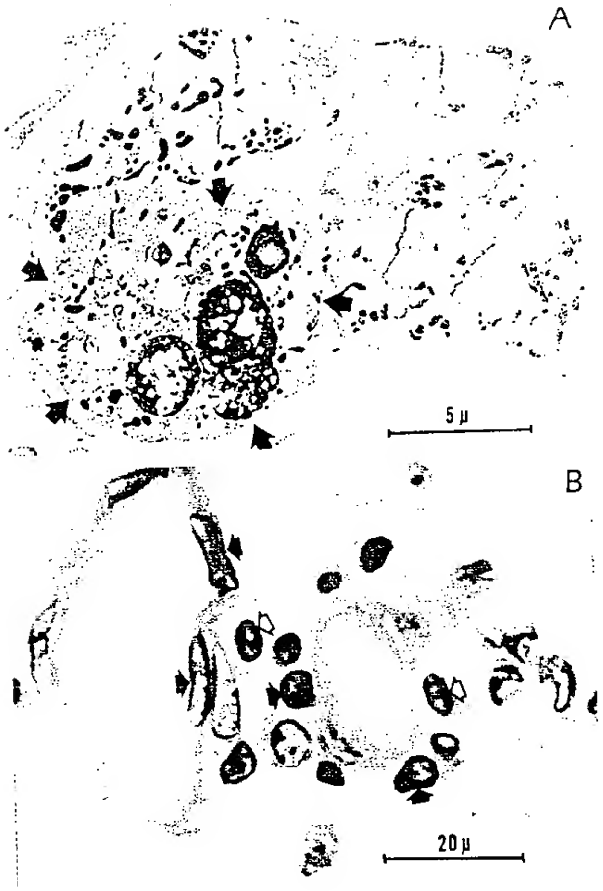


Figure 11. Examples of apoptosis in the sinus node from two different patients dying with the long QT syndrome are shown here. A. Electron micrograph depicts an apoptotic cell filled with apoptotic bodies, outlined with five **black arrows**. Note the pleomorphic small mitochondria in the adjacent myocytes. B. Apoptosis is shown involving a small nodal artery as well as nodal myocytes. Small **black arrows** mark endothelial cells as well as smooth muscle cells of the arterial wall. **Open arrows** mark two nonapoptotic (blue) cells. See also Figure 12.

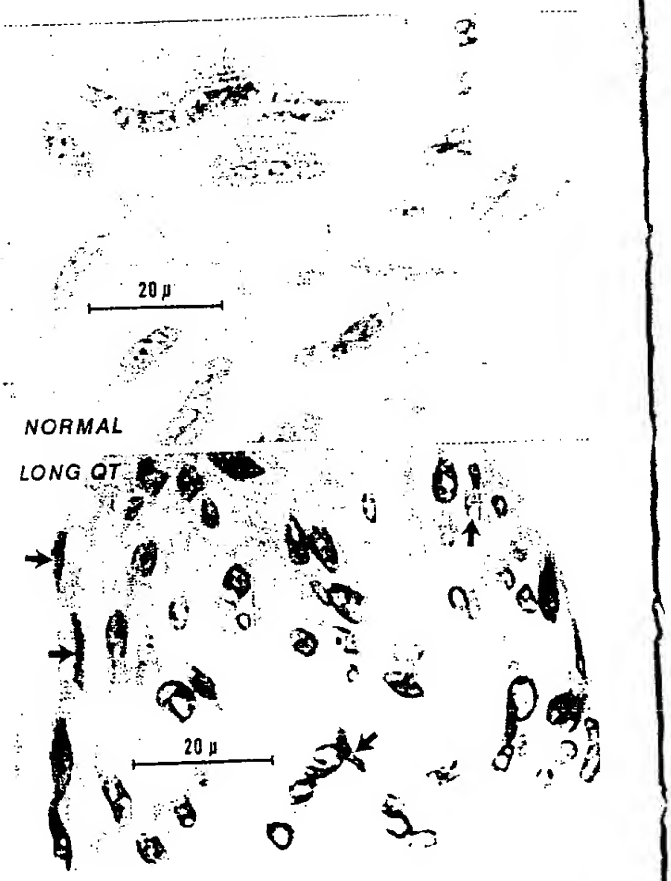


Figure 12. Nonapoptotic (blue) nerve cell nuclei from a normal human sinus node are shown above, for comparison to the extensive apoptosis (brown nuclei) of a ganglion and adjacent nerve from the sinus node of a patient dying with the long QT syndrome (below). **Black arrows** in the long QT example mark the few nonapoptotic (blue) nuclei. See text for discussion.

nerves and ganglia in and near the sinus node, as well as nodal myocytes, is typical of fatal cases of the long QT syndrome (45), and that degeneration of both the myocytes and neural structures is mediated by apoptosis.

The puzzling association of either congenital deafness (33) or normal hearing (34,35) in different victims of the long QT syndrome was further confused by the finding in some families with the long QT syndrome that one symptomatic sibling was deaf but another symptomatic sibling (same family) heard normally (47,48). Recent genetic studies have clarified this matter, as well as how and why delayed ventricular repolarization coexisted with congenital deafness at all, by the demonstration of a common abnormality of cellular potassium channel function affecting both the ear and the heart, caused by a genetic mutation (49,50). This discovery has still broader significance for understanding the pathogenesis of many other arrhythmias and causes for electrical instability of the heart (51).

## APOPTOSIS IN OTHER FORMS OF CARDIAC DISEASE

In most examples of apoptosis involving the heart that I have studied, there is concomitant involvement of not only myocytes but also local small coronary arteries, neural structures, and fibroblasts. This makes pragmatic sense to a point. The local nerves lose much of their useful value if the accompanying myocytes are gone, but one might have some concern from loss of fibroblasts since much of the repair of cell death foci (either apoptotic or necrotic) is by local fibrosis. However, the apoptotic destruction is incomplete in any such focus, leaving not only surviving fibroblasts but also some myocytes.

Apoptosis contributes to focal disease in the endothelium, the tunica media, and even adventitia of both small

and large coronary arteries. It is unclear how often this is a factor of major significance in most examples of coronary atherosclerosis, in part because the multiple intertwined participation by platelets, certain lipids, and increasingly suspected viruses and bacteria, plus the varied response by local endothelium, smooth muscle cells, and invasion of macrophages. It is reasonable to assume that any of these and many other assaults upon coronary artery integrity will in one way or another include a role for apoptosis.

Cardiomyopathy is a disappointingly nebulous term or diagnosis, representing as it does virtually any form of muscle damage in the heart, in many classifications including both known diseases (scleroderma, amyloidosis, and so forth) as well as cases with unexplained etiology ("idiopathic"). Nevertheless, it has become an established diagnostic refuge when managed care or other regulations require such a term, eg, in cases coming to cardiac transplantation. It is probable that cardiomyopathy of any cause at some stage involves apoptosis, much as is the case for coronary disease. Usefulness of this generalization is illustrated when one applies the concept to the broad spectrum of congenital heart diseases (52). Predictably, apoptosis will be found to play some role, minor or major, in every form of heart disease in which it is sought. And we will be measurably more enlightened, if for no other reason than recognizing that necrosis is no longer the only legitimate interpretation for death of cells in the heart.

## HEURISTIC SUMMARIZING THOUGHTS

In addition to understanding the important morphologic and biochemical features of apoptosis there are important clinical considerations to be made when apoptosis affects the heart. Although most of the following examples relate especially to the role of apoptosis in acute myocardial infarction, they are also useful in thinking about clinical presentations of many other cardiac diseases.

### *Chest Pain*

It is currently believed that angina pectoris begins with the effect of an acidic milieu around sensory nerve terminals in the myocardium (53). In view of the fact that apoptosis does not produce such a milieu, while necrosis conspicuously does, one may deduce that apoptotic death of myocytes would not be perceived as painful. But there is different evidence that damage to an important coronary chemoreceptor normally present in the heart may intersect the afferent neural pathway by which nociceptive signals are transmitted to the conscious brain (54). Thus, there are at least two totally different mechanisms by which so-called silent angina or silent myocardial infarction can be explained.

### *Electrical Instability and Sudden Death*

Both apoptosis and necrosis can and do affect myocytes in the entire cardiac conduction system as well as nerves and ganglia within the heart. And because apoptotic and necrotic myocytes or neural structures are equally dead, one could reason that the harmful effects on cardiac electrical instability would be the same. However, the characteristic inflammatory response in necrosis includes major secondary consequences such as production of an acidic environment plus the noxious influences attending an invasion of neutrophils and lymphocytes. Thus, a ventricular myocardial focus dying predominantly by necrosis would be more likely to serve transiently as an abnormal center for an automatic form of arrhythmia, but an apoptotic focus would be less likely to do so. A reentrant arrhythmia, by contrast, could emerge around a central "silent" or nonconducting focus with either apoptotic or necrotic myocytes. Direct damage to the sinus node or the AV node by either apoptosis or necrosis may lead to either an automatic or reentrant arrhythmia, just as may evolve in ventricular myocardium. Cell death of either type in the His bundle would be expected to alter conduction and cause either partial or complete heart block, depending upon the extent of injury. Whether arrhythmias or conduction disturbances so visualized would end in sudden death additionally depends in a major way upon the presence or absence of many different associated factors, all of which coexist in most cases largely as a matter of chance (55).

### *Electrocardiographic Effects*

Nearly all of our present knowledge about changes in the ECG during cardiac diseases is based upon an appreciation of necrosis and not apoptosis. It is probable but not certain that configuration of the QRS complex or the form of the P wave would be similarly changed during either apoptosis and necrosis, as would the PR interval. However, the so-called "injury current" effects (ST-segment elevation or depression, T-wave configuration) may be quite different. This again is attributable to the special nature of inflammation accompanying every focus of necrosis but absent in apoptosis. Since most clinical thinking during suspected or known myocardial infarction anticipates concomitant changes in both the QRS complex and the ST-T configurations, multiple apoptotic foci (eg, in TTP) may easily evade clinical recognition from the ECG. Cases of silent myocardial infarction may be similarly confusing, particularly if only subtle or minor QRS changes occur. This difficulty heightens the urgency of need for some method of detecting and quantifying apoptotic cell death in the heart in vivo.

### *"Calculated" Versus Real Loss of Myocardium*

Current practice continues to assess myocardial loss with measurement of serum markers, most recently the isoforms of CK and now more especially one or more of the

troponins. But as discussed earlier concerning acute myocardial infarction, this practice is seriously flawed by its inescapable failure to recognize or quantify cell loss due to apoptosis. Newer forms of echocardiographic or magnetic resonance imaging may help resolve this problem, and it is predictable that careful studies will find substantial differences between interpretation from these imaging systems and from information provided by serum marker diagnosis. It should also be noted that myocardial damage other than that caused by myocardial infarction (eg, chest contusion, or myocarditis) will also always be underestimated, often by a large margin, when serum marker diagnosis is utilized. Iatrogenic conditions (after angioplasty or thrombolysis or coronary surgery) require this same recognition of the limited value offered by serum marker diagnosis.

### *"Ischemic Preconditioning" Revisited*

In 1986 Murry, Jennings, and Reimer (56) published a heuristic and novel finding that brief bouts of experimental myocardial ischemia resulted in a surprisingly smaller infarction than the infarct produced by a single prolonged or permanent interruption of flow to a similar portion of myocardium. This tantalizing discovery has led to intense investigative interest because of the promise it held for the human condition, with a recent review examining the current status of this seeming paradox (57). Both in the original paper and the recent review, the infarction was defined by demonstrable *necrosis*. As indicated in the 1998 review, there is currently no consensus explanation as to how or why such protection could occur (57).

There are many reasons for hesitating to suggest that the initial premise may be incorrect. However, a main flaw in the original and most subsequent studies is the omission of due consideration for the role apoptosis is now known to play in every human and probably most experimental myocardial infarctions, as was discussed earlier (17,18). Furthermore, there have been important works demonstrating dose-dependent induction of apoptosis in a variety of tissues by graded responses to several different forms of injury, and lesser injuries caused apoptosis while more severe injuries (same tissues, same types of injury) caused necrosis (58,59). The analogy to brief and then sustained forms of coronary occlusion is apparent.

Other studies have shown that brief periods of experimental ischemia in rat kidneys cause apoptosis but prolonged ischemia produced necrosis (60). Gottlieb et al (61) have shown that reperfusion and not ischemia alone led to apoptosis in rat cardiomyocytes, and later explained the roles of pH, vacuolar proton ATPase, and apoptosis in preconditioning of rabbit cardiomyocytes (62). Piot et al (63) postulated that ischemic preconditioning in rat hearts might be due to a reduction in the amount of apoptosis they could later demonstrate after prolonged coronary occlusion; although they described less apoptosis after prolonged coronary occlusion in preconditioned hearts, they did not assess how much apoptosis may have been produced during each of the periods of brief preconditioning ischemia. Laskey (64) made the same error by studying possible beneficial effects of brief ischemia produced in human subjects during percutaneous transluminal coronary angioplasty, concluding that this human ischemic preconditioning was beneficial in reducing myocardial necrosis because there was less creatine kinase elevation in his high-risk population, but neglecting to consider that necrosis is not the only form of myocyte death in human hearts.

It remains disturbingly plausible that the simple reason why less myocardial destruction (by either apoptosis or necrosis) is found after preconditioning is because a substantial amount of destruction by apoptosis per se had already been produced by putatively protective episodes of repeated brief coronary occlusions. This is certainly consonant with the experiments in rat kidney (60) as well as the evidence that brief or less intense forms of any injurious influence regularly caused apoptosis whereas more severe injuries caused necrosis (58,59).

The magnitude of immediate relevance for this hypothetical suggestion is illustrated by three points: first, that the entire subject of ischemic preconditioning is still considered a "paradox" (57) despite more than a decade of intensive investigation by countless research programs, with no consensus as to its explanation; second, the original (56) and current (57) definition of the area at risk and/or infarction depends upon identification of "necrosis," and the presence or absence or size of the necrotic infarct was and still is the gold standard for such studies; and third, without due consideration of any extent of myocardial loss due to apoptosis during preconditioning, there is the disappointing and haunting possibility that the entire concept of protection by preischemic conditioning is an imaginative and appealing chimera.

### *Necessary Reconsiderations Regarding Diagnosis, Triage, and Prognosis in Cardiac Diseases*

Until some in vivo method for recognizing and quantifying death of myocytes by apoptosis becomes available, significant problems will remain in accurately assessing myocardial damage during and following angioplasty or thrombolysis or coronary surgery, as well as the development and evolution of acute myocardial infarction. Furthermore, a number of inescapable problems attending some of these circumstances will remain unrecognized or misinterpreted. For example, during either angioplasty or thrombolysis (now gaining the ugly but appropriate name of clot-busting), it is a predictable corollary that fragments of plaque debris or components of the "bust-

"ed" clot will be hurled downstream to occlude many terminal small coronary branches. It is comforting to think that the clot fragments somehow dissolve, although this is uncertain, but it is more likely that the plaque fragments do cause permanent small coronary obstruction. Some of these small coronary branches are the same anatomical substrate upon which gradually developing collateral circulation would later have evolved. One may protest that collateral circulation is not required if the main coronary occlusion was eliminated and that its patency was assured indefinitely. That is too often not the case in the target artery, but the same occluded small coronary arteries are also the ones necessary to have as a route of collateral circulation in the event of later occlusion of nearby major coronary arteries other than the original targeted one. Thus, whether dealing clinically with either the consequences of an original coronary occlusion, or the iatrogenic effects inescapable in treating it with modern methods, or anticipating the possible later effect of a different coronary occlusion in the same patient, the need for more precise methods for a true assessment of the extent of myocardial damage remains a pressing issue, and such an assessment must include both apoptotic and necrotic cell death.

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